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The effect of feeding diets containing avoparcin on the excretion of salmonellas by chickens experimentally infected with natural sources of salmonella organisms

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SUMMARY

Chickens were readily infected with salmonella organisms when fed diets containing unsterilized bone-meal or provided with drinking water containing a suspension of natural salmonella infected chicken faeces. When fed diets containing avoparcin at concentrations of 10 or 100 mg/kg chickens infected in these ways excreted larger numbers of salmonellas for longer periods than did chickens fed a non-medicated diet.

INTRODUCTION

Smith & Tucker (1978, 1980) found that the feeding of diets containing avoparcin, an antibiotic used for growth promotion of farm animals, favoured the colonization of the chicken alimentary tract by *Salmonella typhimurium*. Chickens fed a diet containing avoparcin usually excreted larger numbers of salmonellas for longer periods of time than unmedicated chickens. They also found that smaller numbers of salmonellas were required to initiate infection and once established infection spread more rapidly in medicated than in non-medicated chickens. Similar results were obtained with four other salmonella serotypes and when experiments were carried out using four breeds of chickens and one of turkey, four different diets and when wire mesh flooring was replaced with deep litter.

Avoparcin was also found by other workers to increase excretion of salmonellas by chickens (Matthes, Leuchtenberger & Loliger, 1981). Other results have been equivocal. In field observations Smith & Green (1980) found no such effect but the chickens involved in their study received a variety of other chemotherapeutic agents during the course of the experiments. Gustafson, Beck & Kobland (1982) found that avoparcin promoted the colonization of the chicken gut in one out of three experiments in which chickens were either infected orally or via the drinking water by broth cultures of a strain of *S. typhimurium*.

Wherever experimental infection has been used this has involved broth cultures of salmonellas. Because cultured salmonella organisms may behave differently from 'wild' salmonellas it was decided to see what effect the feeding of diets containing avoparcin might have on naturally infected chickens. The two most important sources whereby chickens acquire such infection are by the consumption of diets containing nutritional additives, particularly protein supplements, that

are contaminated with salmonellas or by exposure to the faeces of chickens, or other animals involved in natural outbreaks of salmonella infection. Consequently, in our studies we employed as sources of infection bone-meal known to be salmonella contaminated and the faeces of broiler chickens from a subclinical outbreak of *S. montevideo* infection. The results are reported in this paper.

MATERIALS AND METHODS

Chickens

The kind of chickens, their management and diet have been described previously (Smith & Tucker, 1975). For the experiment involving feeding bone meals all chickens were kept on wire floored pens until 100 days of age after which they were housed on slatted floors. Chickens in the other experiments were kept on deep litter.

Method of infecting chickens

Salmonella-contaminated bone-meal was obtained from retail outlets (Smith *et al.* 1982). Six samples were used; the salmonella serotypes they contained are listed in Table 1.

Table 1. *Salmonella serotypes isolated from bone-meal samples used*

Bone-meal sample number	Serotypes isolated
1	*S. senftenberg*, *S. newport*, *S. derby*
2	*S. senftenberg*, *S. newport*, *S. lexington*, *S. typhimurium* phage type 161
3	*S. agona*, *S. anatum*
4	*S. agona*
5	*S. derby*, *S. schwarzengrund*, *S. mbandaka*, untyped 3, 10:e,h.
6	*S. lexington*

In both experiments in which bone-meal was used as the source of salmonellas, groups of chickens were infected by mixing the bone-meals in the diet at a level of 5%. In the first experiment the period of exposure to salmonella infection was 0–24 days of age. In the second experiment groups of chickens were given bone-meal in the diet for different periods. Bone-meals 1, 2 and 3 were included in the diet from 4 to 14 days of age, samples 4 and 5 from 4 to 21 days and sample 6 from 4 to 25 days.

A composite sample of faeces containing *S. montevideo* was obtained from a healthy commercial flock. The faeces sample was mixed with the drinking water to provide the chickens with water containing a *S. montevideo* count of 10 viable bacteria per ml as calculated by the most-probable-number method (Cruickshank, 1970). This salmonella contaminated water was provided for a period of 14 days and was changed every 48 h.

Detection of salmonella excretion by experimentally infected chickens

This was carried out using modifications to the methods of Smith & Tucker (1975). Direct culture of cloacal swabs on to Desoxycholate Citrate agar (Oxoid,

CM163) and Brilliant Green agar (Oxoid, CM263) was used in conjunction with enrichment culture using selenite broth (Oxoid, CM39). Colonies resembling those of salmonella were identified by slide-agglutination using commercially available antisera (Wellcome).

RESULTS

The faecal excretion of salmonellas by chickens fed a diet containing bone-meal and avoparcin

The results of examining the faeces of groups of ten chickens infected by providing them with food containing bone-meal and containing avoparcin at 0, 10 or 100 mg/kg are presented in Tables 2 and 3. In the experiment summarized in Table 2 groups of chickens were fed infected food from 0 days of age until 24 days, when the bone-meal was discontinued. Since the bone-meal was supplied to each group for the same period and the results obtained were similar, the results have been pooled together. In the experiment whose results are shown in Table 3, groups of chickens were provided with infected food from 4 days of age for different periods. The results obtained from the groups fed bone-meal samples 1, 2 and 3 and also 4 and 5 were pooled because the samples had been included in the diet for the same length of time. The results were similar for each bone-meal studied. Avoparcin was included in the diets throughout both experiments.

The results were generally similar for both experiments. Infection of the chickens by salmonellas occurred rapidly and the levels of infection increased in all groups until the bone-meals were withdrawn. The presence or absence of avoparcin in the diet seemed to have little effect on the speed with which groups of chickens became infected. In several groups all the chickens were infected by the time the bone-meal was discontinued from the diet. After withdrawal of the bone-meal the levels of infection gradually declined. This decline was faster in the groups which received no avoparcin, with the result that after a few weeks higher levels of salmonella infection were found in the groups fed avoparcin than in those receiving an unmedicated diet. This situation lasted until the end of the experiment. When the chickens were killed, similar results were found on culturing the caecal contents.

Results from groups fed avoparcin at the 10 and 100 mg/kg levels were compared with the groups fed no avoparcin using the χ^2 test. In all comparisons the differences observed were statistically significant, i.e. $P < 0{\cdot}001$.

The faecal excretion of salmonella organisms by chickens provided with drinking water containing a suspension of Salmonella montevideo *infected faeces.*

The results of culturing the faeces of groups of 30 chickens given drinking water containing *S. montevideo* infected faeces are shown in Table 4. Chickens were exposed to infection from 0 to 14 days or from 7 to 21 days of age. *S. montevideo* was isolated from the faeces of chickens from all groups as early as 4 days after the infected water was provided. After the source of infection was removed, salmonellas were isolated from fewer unmedicated chickens than from those fed 10 or 100 mg/kg avoparcin in the diet. Culture of caecal contents at the end of the experiment largely reflected these results.

The χ^2 test was again used for analysis of the results. The same comparisons were made and these were found to be statistically significant, i.e. $P < 0{\cdot}001$.

Table 2. *The isolation of salmonella organisms from the faeces of groups of* 10 *chickens fed on diets containing* 5% *unsterilized bonemeal from* 0–24 *days of age*

Bonemeal samples 1–6†	Avoparcin conc. (mg/kg)	% of chickens from which salmonella organisms were isolated at days																							
		6	8	11	14	18	21	27	34	41	48	55	62	68	76	83	90	97	104	111	118	125	132	139	147	Caeca*
	0	6	37	41	52	60	70	82	61	87	82	74	61	46	23	8	13	36	24	12	10	9	9	9	3	10
	10	0	2	5	13	30	56	98	95	98	92	97	95	82	66	23	17	64	47	41	51	42	39	41	47	53
	100	9	15	17	15	69	88	97	98	100	100	100	98	97	92	84	86	90	90	80	82	62	66	69	56	95

* Results of examining caecal contents when the chickens were killed at the end of the experiment.
† Pooled results for 60 chickens.

Table 4. *The isolation of salmonella organisms from the faeces of groups of* 30 *chickens given drinking water containing a suspension of* Salmonella montevideo *infected faeces and fed diets containing different levels of avoparcin*

Period of exposure to infection	Avoparcin conc. (mg/kg)	% of chickens from which salmonellas were isolated at days												
		0*	4	7	11	14	18	21	28	35	42	47	55	Caeca
0–14 days	0	0	23	23	20	17	23	23	10	3	3	10	3	0
	10	0	40	73	60	30	33	23	47	50	20	10	10	20
	100	0	43	47	50	67	37	50	66	60	83	27	57	90
7–21 days	0	0	7	17	17	7	17	3	14	0	0	3	—	3
	10	0	3	7	13	23	50	33	17	13	17	13	—	7
	100	0	33	80	97	93	97	80	83	67	60	37	—	90

For other details and abbreviations see Table 2.
* Number of days after 1st day of provision of infected water.

Table 3. *The isolation of salmonella organisms from the faeces of groups of* 10 *chickens fed on diets containing* 5% *unsterilized bone-meal for different periods*

No. of bone-meal sample contained in diet	Avoparcin conc. (mg/kg)	% of chickens from which salmonella organisms were isolated at days																				
		4	6	8	10	14	17	23	30	37	44	51	58	65	72	79	86	93	100	107	115	Caeca*
1, 2, 3†	0	0	0	36	39	48	56	74	85	48	67	15	74	83	15	19	12	16	8	12	12	12
	10	0	0	33	63	70	93	100	93	97	90	90	33	60	37	90	59	59	52	52	31	66
	100	0	10	93	97	100	90	100	93	83	93	90	45	86	41	97	79	79	59	72	79	97
4, 5‡	0	0	0	65	25	30	65	100	89	47	26	21	21	6	0	6	6	0	0	0	0	6
	10	0	5	68	44	89	78	94	89	78	89	28	28	67	33	78	56	50	33	56	78	89
	100	0	17	89	100	100	94	100	100	94	94	50	50	89	50	94	53	82	88	70	77	82
6	0	0	0	90	100	70	80	100	90	50	100	60	20	30	30	20	40	20	0	0	10	10
	10	0	0	20	20	100	100	100	100	100	90	90	60	70	60	100	80	80	60	60	40	30
	100	0	40	100	100	100	100	100	100	100	90	90	70	100	50	100	100	100	90	80	90	100

* Results of examining caecal contents when the chickens were killed at the end of the experiment.

† Pooled results from experiments in which chickens were fed bone-meals 1, 2 and 3, involving 30 chickens.

‡ Pooled results from experiments in which chickens were fed bone-meals 4 and 5, involving 20 chickens.

DISCUSSION

Earlier studies (Smith & Tucker, 1978, 1980) showed that avoparcin increased the excretion of different salmonella serotypes by chickens reared under a variety of conditions. Because chickens were infected with bacterial cultures in those experiments it is conceivable that different results might have been obtained by using more natural sources of salmonella infection.

Under natural conditions chickens are usually infected from contaminated protein feed supplements or directly from other chickens. In our experiments we have attempted to mimic these routes by mixing unsterilized bone-meal containing salmonellas with the food or by making a suspension of salmonella infected chicken faeces in the drinking water. Chickens were readily infected by these methods. Avoparcin incorporated in the diet at 10 or 100 mg/kg generally increased excretion of salmonellas by these chickens. This occurred whether the chickens were infected at 0 or 4 days of age.

Since these results support the studies of Smith and Tucker it now seems likely that in most situations in which chickens become infected the use of avoparcin as a growth promoting antibiotic would increase the faecal excretion of salmonellas.

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